Final Scientific Report

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BARD Project Number: IS 3733R

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Project Title:

Increasing Starch Accumulation via Genetic Modification of ADP-glucose Pyrophosphorylase

Investigators	**Institutions**
Principal Investigator (PI):	
Arthur Schaffer	ARO
Co-Principal Investigator (Co-PI):	
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Ilan Levin	ARO
Marina Petreikov	ARO

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order**:**

Budget: IS: $ US: $ Total: $

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged		9	2	11
Submitted, in review, in preparation	1			1
Invited review papers		7		7
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1		1
Longer Visits (Sabbaticals)				

Description Cooperation: Cooperation between the two research groups consisted of transfer of biological material and protocols, a three week visit by Dr. Petreikov for training in techniques in the Preiss lab, as well as a joint publication describing the novel observation of activity of an AGPase large subunit. During the first year the US lab transferred to the Israeli lab clones of the small and large subunits of the potato ADP-Glc PPase and during the second year Dr. Marina Petreikov enjoyed an extended visit in the US lab where she acquired the techniques of heterologous bacterial expression of the different AGPase subunits. This cooperation led to the joint publication from the Schaffer and Preiss labs of Petreikov et al (submitted). In addition, e-mail correspondence including transfer of expertise and protocols, as well as Rosh Hashanah greetings, was carried out throughout the project.

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Abstract

The overall objective of the research project was to utilize biochemical insights together with both classical and molecular genetic strategies to improve tomato starch accumulation. The proposal was based on the observation that the transient starch accumulation in the immature fruit serves as a reservoir for carbohydrate and soluble sugar content in the mature fruit, thereby impacting on fruit quality. The general objectives were to optimize AGPase function and activity in developing fruit in order to increase its transient starch levels. The specific research objectives were to: a) perform directed molecular evolution of the limiting enzyme of starch synthesis, AGPase, focussing on the interaction of its regulatory and catalytic subunits; b) determine the mode of action of the recently identified allelic variant for the regulatory subunit in tomato fruit that leads to increased AGPase activity and hence starch content.

During the course of the research project major advances were made in understanding the interaction of the small and large subunits of AGPase, in particular the regulatory roles of the different large subunits, in determining starch synthesis. The research was performed using various experimental systems, including bacteria and Arabidopsis, potato and tomato, allowing for broad and meaningful conclusions to be drawn. A novel discovery was that one of the large subunits of tomato AGPase is functional as a monomer. A dozen publications describing the research were published in leading biochemical and horticultural journals.

The research results clearly indicated that increasing AGPase activity temporally in the developing fruit increase the starch reservoir and, subsequently, the fruit sugar content. This was shown by a comparison of the carbohydrate balance in near-isogenic tomato lines differing in a gene encoding for the fruit-specific large subunit (LS1). The research also revealed that the increase in AGPase activity is due to a temporal extension of LS1 gene expression in the developing fruit which in turn stabilizes the limiting heterotetrameric enzyme, leading to sustained starch synthesis. This genetic variation can successfully be utilized in the breeding of high quality tomatoes.

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Achievements and impacts

ADP-glucose pyrophosphorylase (ADP-Glc PPase) catalyzes the regulatory step in the pathway for synthesis of bacterial glycogen and starch in plants, thereby allowing bacterial glycogen synthesis to serve as a model for plant starch synthesis. A major goal of the project was to study the function of key domains of the AGPase enzymes, as well as the interactions between the largely catalytic small subunit and the primarily regulatory large subunit. This was performed using various strategies, including preparation of chimeric enzymes incorporating different domains within a single subunit, as well as combining different subunits and characterizing their activities and regulatory properties.

In one study chimeric enzymes were constructed from cyanobacterial and plant enzymes to obtain homo- and heterotetrameric chimeric proteins. Characterization of these forms showed that the N-terminus determines stability and regulatory redox-dependent properties. The chimeric forms exhibited intermediate 3-phosphoglycerate activation properties with respect to the wild-type homotetrameric enzymes indicating that the interaction between the putative N- and C-domains determine the affinity for the activator. Characterization of the chimeric heterotetramers showed the functionality of the large subunit, mainly in modulating regulation of the enzyme by the coordinate action of 3-phosphoglycerate and inorganic orthophosphate. Furthermore, the US group utilized a novel strategy to determine regions critical for AGPase activity by inserting a variety of pentapeptides in the bacterial enzyme and characterizing its effect on enzyme activity. These results are described in the paper by Ballicora et al., 2007.

The US lab also characterized in detail the hybrid enzymes made of heterotetrameric potato small unit and the different Arabidopsis large subunits. Their results are described in the paper by Ventriglia et al., 2007. The results show that the different large subunits of Arabidopsis confer unique regulatory properties to the heterotetramer and, most significantly, these properties are consistent with the functions of the enzymes in their respective source and sink tissues. In parallel, the Israeli lab isolated and expressed the different tomato large subunits in E. coli, together with the tomato small subunit, and characterized the activity and regulatory properties. The different large subunits of tomato are expressed either in the fruit

sink or the source leaves and the results indicate that the different tetrameric enzymes indeed have distinct regulatory characteristics which can be related to their physiological and tissue specific roles.

The research also pointed to active catalytic roles of the plant large subunits, which is a novel conclusion with possible future applications in starch synthesis. The US group showed that in Arabidopsis, two large subunits APL1 and APL2, besides their regulatory role, have catalytic activity. Heterotetramers formed by combinations of a mutant non-catalytic small subunit APS1 and the large subunits showed that APL1 and APL2 exhibited ADP-Glc PPase activity with distinctive sensitivities to the allosteric activator (3-PGA). Results from transgenic Arabidopsis plants with APL1 and APL2 having catalytic activity indicated that the large subunit may contribute to ADP-Glucose synthesis *in planta*.

This novel observation was carried further in the Israeli lab who showed that one of the tomato large subunits (leaf-specific LS3) was active even in the absence of an inactive small subunit, and that the LS3 was active as a monomer rather than as a homotetramer. Although the activity of this monomeric large subunit is low and has poor regulatory characteristics, this discovery opens new research strategies in starch synthesis in plants.

One of the major goals of the research project was to determine the feasibility of modulating starch content in the developing tomato fruit in order to impact on fruit carbohydrate balance and sugar content. To this end we studied in detail the contribution of a wild species allele for the AGPase large subunit LS1 in cultivated tomato plants. Tomato plants (*Solanum lycopersicum*) harboring the allele for the AGPase large subunit (*AgpL1*) derived from the wild species *Solanum habrochaites* (*AgpL1*^{H}) are characterized by higher AGPase activity and increased starch content in the immature fruit, as well as higher soluble sugars in the mature fruit following the breakdown of the transient starch, as compared to fruit from plants harboring the cultivated tomato allele (*AgpL1*^{E}). These results are described in Petreikov et al, 2009. Comparisons of AGPase subunit gene expression and protein levels during fruit development indicate that the increase in AGPase activity correlates with a prolonged expression of the *AgpL1* gene in the *AgpL1*^{H} high starch line, leading to an extended presence of the L1 protein. The S1 (small subunit)

protein also remained for an extended period of fruit development in the *AgpL1*^{H} fruit, linked to the presence of the L1 protein. There were no discernible differences between the kinetic characteristics of the partially purified AGPase-L1^{E} and AGPase-L1^{H} enzymes. The results indicate that the increased activity of AGPase in the *AgpL1*^{H} tomatoes is due to the extended expression of the regulatory L1 and to the subsequent stability of the heterotetramer in the presence of the L1 protein, implying a role for the large subunit not only in the allosteric control of AGPase activity but also in the stability of the AGPase L1-S1 heterotetramer. The introgression line of *S. lycopersicum* containing the wild species *AgpL1*^{H} allele is a novel example of transgressive heterosis in which the hybrid multimeric enzyme shows higher activity due to a modulated temporal expression of one of the subunits.

Description of the cooperation

Cooperation between the two research groups consisted of transfer of biological material and protocols, a three week visit by Dr. Petreikov for training in techniques in the Preiss lab, as well as a joint publication describing the novel observation of activity of an AGPase large subunit. During the first year the US lab transferred to the Israeli lab clones of the small and large subunits of the potato ADP-Glc PPase and during the second year Dr. Marina Petreikov enjoyed an extended visit in the US lab where she acquired the techniques of heterologous bacterial expression of the different AGPase subunits. This cooperation led to the joint publication from the Schaffer and Preiss labs of Petreikov et al (submitted). In addition, e-mail correspondence including transfer of expertise and protocols, as well as Rosh Hashanah greetings, was carried out throughout the project.

Publications:

12 papers have been written describing research partially funded by the BARD project:

Bejar, C., Jin, X., Ballicora, M. A., and Preiss, J. (2006a) Molecular architecture of the glc-1-P site in ADP-glc pyrophosphorylases. J Biol Chem 281:40473-40484.

Bejar, C., Ballicora, M. A., Iglesias, A. A. and Preiss, J. (2006b) ADP-glucose pyrophosphorylase's N-terminus: structural role in allosteric regulation. Biochem. Biophys. Research Commun. 343: 216-221.

Iglesias, A. A., Ballicora, M. A., Sesma J. I. and Preiss, J. (2006) Domain swapping between a cyanobacterial and a plant subunit ADP-pyrophosphorylase. Plant and Cell Physiology. 47:523-530.

Yep, A., Ballicora, M.A. and Preiss, J (2006) The ADP-glucose binding site of Escherichia coli Glycogen Synthase. Arch. Biochem. And Biophysics 453:188-196.

Petreikov, M. Shen, S. Yeselson, Y. Levin, I. Bar, M. and Schaffer A. A. (2006) Temporally extended gene expression of the ADP-glu pyrophosphorylase large subunit (AgpL1) leads to increased enzyme activity in developing tomato fruit. Planta 224:1465-1479.

Ballicora MA, Erben ED, Yazaki T, Bertolo AL, Demonte AM, Schmidt JR, Aleanzi M, Bejar CM, Figueroa CM, Fusari CM, Iglesias AA, Preiss J. (2007). Identification of regions critically affecting kinetics and allosteric regulation of the Escherichia coli ADP-glucose pyrophosphorylase by modeling and pentapeptide-scanning mutagenesis. J Bacteriol. 189: 5325-33.

Ventriglia T, Ballicora MA, Crevillen P, Preiss J, Romero JM. (2007). Regulatory properties of potato-Arabidopsis hybrid ADP-glucose pyrophosphorylase. Plant Cell Physiology 48:875-880.

Ventriglia T, Kuhn ML, Ruíz MT, Pedro MR, Valverde F, Ballicora MA, Preiss J, Romero JM (2008) Two Arabidopsis ADP-Glucose Pyrophosphorylase large subunits (APL1 and APL2) are catalytic. Plant Physiol. 148: 65-76.

Sheng F, Jia X, Jep J, Preiss J, Geiger J (2009) The crystal structures of the open and catalytically competent closed conformation of Escherichia coli glycogen synthase. J. Biol. Chem. 284, 17796-17807.

Sheng F, Yep A, Fang L, Preiss J, Geiger J (2009) Oligosaccharide binding in escherichia coli glycogen synthase. Biochemistry 48:10089-10097.

Petreikov Marina, Yeselson Lena, Shen Shmuel, Efrat Ari, Bar Moshe, Levin Ilan, Schaffer Arthur A. (2009) Carbohydrate Balance and Accumulation during Development of Near-Isogenic Tomato Lines Differing in the AGPase-L1 Allele. J. Amer. Soc. Hort. Sci. 134:134–140.

Petreikov M, Eisenstein M, Yeselson Y, Preiss J and Schaffer AA. The tomato AGPase large subunit isoforms confer differences in activities to the heterotetrameric enzyme and the LeL3 subunit itself is active as a monomer (submitted to Plant Physiology).

In addition, seven reviews have been written.

Preiss, J. (2008) Starch Biosynthesis in Plants. In Wiley Encyclopedia of Chemical Biology. John Wiley and Sons. DOI:10.1002/9780470048672.wecb191.

Preiss J. (2009) Starch Biosynthesis in Plants, Volume 4, pp 362-376, in Wiley Encyclopedia of Chemical Biology, John Wiley & Sons, Hoboken.

Preiss, J. (2009) "Glycogen Biosynthesis", in *The Encyclopedia of Microbiology*, 3rd Edition, edited by Moselio Schaechter, Oxford: Elsevier, UK, pp. 145-158.

J.M. Shively, G. C. Cannon, S. Heinhorst, J.A. Fuerst, D.A. Bryant, E. Gantt, J.A. Maupin-Furlow, D. Schüler, F. Pfeifer, R. Docampo, C. Dahl, J. Preiss, A. Steinbüchel and B.A. Federici. (2009) Intracellular Stuctures of Prokaryotes: Inclusions, Compartments and Assemblages. In Encyclopedia of Microbiology, 3rd Edition. (M. Schaechter, Ed.) Elsevier, Inc., Oxford, UK. Pp. 404-424.

Preiss, J. (2009) Biochemistry and Molecular Biology of Starch Biosynthesis. IN: Starch: Chemistry and Technology (3rd edition) R.L. Whistler and J. BeMiller (eds.) Elsevier, Inc., Oxford, UK, pp 83-147.

Preiss, J. (2009) Biochemistry and Molecular Biology of Glycogen Biosynthesis in Bacteria and Mammals and Starch synthesis in Plants. In Comprehensive Natural Products Chemistry II, Clair Byrne, Developmental Editor Elsevier, Inc., Oxford, UK..

Preiss, J. Glycogen Biosynthesis and Regulation. *In* A. Böck, R. Curtiss III, J. B. Kaper, P. D. Karp, F. C. Neidhardt, T. Nyström, J. M. Slauch, and C. L. Squires, and D. Ussery (ed.), EcoSal—*Escherichia coli* and *Salmonella:* cellular and molecular biology. Chapter 4.7.4, http://www.ecosal.org. ASM Press, Washington, D.C.

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Appendix: The following papers are appended:

Bejar, C., Jin, X., Ballicora, M. A., and Preiss, J. (2006a) Molecular architecture of the glc-1-P site in ADP-glc pyrophosphorylases. J Biol Chem 281:40473-40484.

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Ballicora MA, Erben ED, Yazaki T, Bertolo AL, Demonte AM, Schmidt JR, Aleanzi M, Bejar CM, Figueroa CM, Fusari CM, Iglesias AA, Preiss J. (2007). Identification of regions critically affecting kinetics and allosteric regulation of the Escherichia coli ADP-glucose pyrophosphorylase by modeling and pentapeptide-scanning mutagenesis. J Bacteriol. 189: 5325-33.

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